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[54]发明名称 高钙味素及制作

[57]摘要

本发明涉及一种食品调味素及制作，它是一种高钙味素及制作。该发明的生物钙味素有酶解牛骨粉、味精、食盐、糊精、鸟苷酸钠+肌苷酸钠。将上述组分粉碎混合后制成小包装调味素，可用于汤和菜肴中；起到调味，补钙作用。本发明的产品可放人各种的汤料、菜肴中。

# 权利要求书

1、一种高钙味素及制作，其特征在于高钙味素的组份有酶解牛骨粉、味精、食盐、糊精和鸟苷酸钠+肌苷酸钠，其各组份的重量配比为：0·5–1·5：2·225–6·9：0·125–0·39：0·3–1·05：0·048–0·15，其制作过程为：先将味精、食盐、糊精、鸟苷酸钠+肌苷酸钠按比例混合后进行粉碎处理；混合物料再与酶解牛骨粉混合30分钟后即可包装入库。

# 高钙味素及制作

本发明涉及一种食品调味素及制作，它是高钙味素及制作。

现在人们在饮食中不仅求饱、求质量，还不断需要增加调味，使人们在食用时更讲究舒服及口感。为此商家推出各种调味品，汤料、油炸料、调料等等。但这些小料只能是用作在美味家肴中再增添点适合自己口感的佐料，而佐料本身除提供所需味道之外无其它保健功能。随着人们口味的增加，对饮食营养化的要求越来越高，因此，人们更需要在调料中得到各方面的补充。尤其是儿童与老人喜欢饭后喝汤，这样可以使饭菜更好地得到吸收，但我们在自己做汤或冲汤料时都不可能在汤中加放补钙食品，而儿童和老人是最需要加强补钙的对象，这样他们就需要再利用其它途径补充钙物质。

本发明的目的在于提供一种高钙味素及制作，它是一种可放汤和菜肴中的调味品，该调味品可使人们在品尝美味家肴中得到生物钙的补偿。

本发明的技术内容为：高钙味素及制作，它的组份中含有酶解牛骨粉、味精、食盐、糊精、鸟苷酸钠和肌苷酸钠，各组份的重量配比为：0·5–1·5：2·225–6·9：0·125–0·39：0·3–1·05：0·048–0·15；其制作过程为：先将味精、食盐、糊精和鸟苷酸钠+肌苷酸钠做粉碎，然后与酶解牛骨粉混合30分钟即可包装入库。

本发明的特点是：在汤料或调料中添加酶解牛骨粉，可使人们在用饭时得到钙的补充，而不必要再通过其它方式单纯补钙，添加鸟苷酸钠+肌苷酸钠可提高调味品的鲜味，增加口感。本发明适合于小儿佝偻病和老年骨质疏松症。本发明易于

制作，可制成小包装，便于携带，是出门旅游办事自己改善饮食口味的调味品。

下面用实施例说明本发明：

实施例1，取味精250克，食盐14克、糊精40克、鸟苷酸+肌苷酸钠6克混合后进行粉碎处理，将粉碎后混合物加放酶解牛骨粉50克再进行30分钟的混合处理，然后上自动装料机进行小袋包装处理即成产品，在食用时将小包装剪开直接倒入需调味的汤剂或菜肴中进行搅拌即可食用。

实施例2，取味精445克，食盐25克，糊精60克，鸟苷酸钠+肌苷酸钠9·6克混合后进行粉碎然后加酶解牛骨粉100克再次混合30分钟后即可按上述步骤进行包装处理。

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Application No. 03140318.2, filed August 28, 2003; Inventors: XIAN Li-jian, CAO Qi-yuan, LI Yong-qiang, SUN, Jian, and LIU Ran-yi. Assignee: Tumor Prevention and Treatment Center, Zhong-shan University

# A NEW USE OF BRASSINOLIDE TO REVERSE MULTI-DRUG RESISTANCE OF CANCER CELLS

[54] Name of Invention A New Use of Brassinolide to Reverse Multi-Drug Resistance of Cancer Cells

[57] Abstract.

This invention pertains to a new use of brassinolide to reverse multi-drug resistance of cancer cells. Its characteristic feature is that it is a new use of brassinolide to reverse multi-drug resistance of cancer cells. Barssinolide possesses very strong biological activity, it is safe and non-toxic; at low concentrations, when brassinolide itself has no tumor-inhibiting effect, it effectively reverses drug resistance of cancer cells with a very high degree of drug resistance.

# CLAIMS

1. A new use of brassinolide to reverse multi-drug resistance characterized in that it is a new use of brassinolide to reverse multi-drug resistance of cancer cells.

# SPECIFICATION

## A New Use Of Brassinolide To Reverse Multi-Drug Resistance

[Field of Technology]

This invention pertains to a new use of brassinolide to reverse multi-drug resistance.

[Prior Art]

Multi-drug resistance of cancer cells currently remains an unsolved problem that has a direct impact on the treatment and prognosis of cancer patients. Currently there still is no clinically available medication that can overcome multi-drug drug resistance.

Brassinolide is (hereinafter referred to as BL) is by now the latest., most biologically active plant growth regulator, its chemical name is (22R, 23R, 24R)-2α, 3α, 22, 23- tetrahydroxy-β-homo-7-oxy-5α-ergostane-6-one. It was first extracted in the 1970ies by the US scientist Mitchell from rape pollen. It is a steroid, and its biological activity is 1000 times higher than that of other plant growth regulators; it has a pronounced effect already at the extremely low concentration of 0.02-0.03 ppm. It comes from plant pollen, and its biomimetic synthesis process is reliable, hence it is non-toxic. As a plant hormone, it is highly effective, broad-spectrum, with a strong stress resistance, low toxic, safe, and has other advantages; very quickly, it attracted the attention of scientists in many countries, and has become known as the sixth plant hormone. In the 1980ies, its biomemetic synthesis was conducted first in Japan, then in China. Brassinolide contains numerous growth regulators, microelements, amino acids, endogenous plant hormones, etc., and is currently used in agricultural production.

Multi-drug resistance means that cancer cells have developed resistance not only to one drug but at the same time also to other drugs of different structures and with different action mechanisms. Multi-drug resistance is the primary cause of chemotherapy failure and has a grave impact on the treatment and prognosis of cancer patients. Research on overcoming multi-drug resistance has been conducted for more than 20 years both in China and abroad, but so far, there is not a single medication capable of achieving a genuine and effective reversal of multi-drug resistance in cancer.

[Contents of Invention]

The characteristic feature of this invention is that it is a new use of brassinolide to reverse multi-drug resistance of cancer cells. Barssinolide possesses very strong biological activity, it is safe and non-toxic; at low concentrations, when brassinolide itself has no tumor-inhibiting effect, it effectively reverses drug resistance of cancer cells with a very high degree of drug resistance.

This invention can develop a new effective multi-drug resistance reversal agent, thus bringing about a more sensitive chemotherapy, benefiting patients, and achieving a beneficial economic and social effect.

[Explanation of Figures]

Fig. 1 shows the impact of brassinolide [product name Tianfengsu – translator's note] on rhodamine accumulation.

Fig. 2 shows the impact of brassinolide on the catalytic activity of DNA-topoisomerase II (TOPO-II).

Fig. 3 shows the impact of brassinolide on the protein expression of sensitive strains and drug resistant cell strains P53, P21.

[Embodiments]

Experimental method

The cell strain CCRF-VCR1000 is a human T-lymphoblast type of leukemia cell strain of MDR induced by vincristine sulfate (VCR), its corresponding sensitive strain is CCRF-CEM. Using the MIT method, the CCRF-VCR1000 cell drug resistance factor was measured as well as the drug resistance reversal coefficient following the BL effect. A flow cytometer was used to measure the accumulation of the sensitive strain and of the drug resistant strain with regard to the fluorescent stain rhodamine 123 transferred by means of P-170 glucoprotein, as well as the change in stain accumulation inside the drug-resistant strain after BL treatment. The catalytic activity of DNA-topoisomerase II (TOPO-II) was measured by Sulliven's method to study the impact of BL-treatment on the TOPO-II catalytic activity. Sensitive cells were detected by the Western blot method, as was the protein expression of the drug-resistant cells P53 and P21, and the BL impact.

**Results**

**Measuring the drug resistance of CCRF-VCR1000 cells**

The $IC_{50}$ value of CCRF-VCR1000 cells for ADM is 8942 ng/ml, the $IC_{50}$ value of its precursor sensitive strain CCRF-CEM cells for ADM is 58.4 ng/ml; the drug resistance coefficient of CCRF-VCR1000 cells for ADM is 153.1 times.

The $IC_{50}$ value of CCRF-VCR1000 cells for VP-16 is 11408 ng/ml, the $IC_{50}$ value of its precursor sensitive strain CCRF-CEM cells for VP-16 is 104.1 ng/ml; the drug resistance coefficient of CCRF-VCR1000 cells for VP-16 is 55.9 times.

The $IC_{50}$ value of CCRF-VCR1000 cells for VCR is 36554 ng/ml, the $IC_{50}$ value of its precursor sensitive strain CCRF-CEM cells for VCR is 4.5 ng/ml; the drug resistance coefficient of CCRF-VCR1000 cells for VCR is 8123.1 times.

**Measuring the cytotoxic effect of BL on CCRG-CEM cells and CCRF-VCR1000: cells**

In concentrations of 0.001 μg/l, 0.01 μg/l, 0.1 μg/l, 1.0 μg/l or 10.0 μg/l BL does not have a cytotoxic effect on CCRF-VCR1000 cells. In a concentration of 100 μg/l; the sensitive strain inhibition rate is 58.9%, and the drug resistance suppression rate is 40.4%.

**BL's drug resistance reversal effect on CCRF-VCR1000 cells:**

n concentrations of 0.001 μg/l, 0.01 μg/l, 0.1 μg/l, 1.0 μg/l or 10.0 μg/l BL reverses the drug resistance of CCRF-VCR1000 to VCR, and the reversal coefficients are 4.0, 4.6, 7.2, 7.5, and 11.6, respectively.

**BL's influence on rhodamine-123 accumulation in CCRF-VCR1000 cells:**

In the absence of BL, over a period of two hours, the accumulation of rhodamine-123 in CCRF-CEM cells gradually increased over time, while rhodamine-123 accumulation in the drug-resistant CCRF-VCR1000 cells clearly slowed down; the difference was dramatic. After treating CCRF-VCR1000 cells with 10 μg/ml BL for 24 hours, the results measured with a flow cytometer clearly demonstrate that rhodamine 123 accumulation in the cells increased, closing up with the sensitive strain CCRF-CEM graph.

**BL's influence on the catalytic activity of DNA-topoisomerase II (TOPO-II) in sensitive and drug-resistant cells:**

The results of TOP-II catalytic activity measurements demonstrate that there are no marked differences in the TOPO-II catalytic activity of the drug resistant strain, sensitive strain and the drug-resistant strain after BL treatment. (Fig. 2)

**BL's influence on the protein expression of the drug resistance strain cells P53 and P21):**

The drug-resistant strain P53 demonstrated a pronounced increase; after 24 hours of treatment with 10.0 μg/ml BL, the protein expression level of the drug-resistant strain P53 was restored to the sensitive strain level. Under the conditions of this experiment, the protein expression of P21 was not measured (Fig. 3.)

**Conclusions:**

Brassinolide exhibits a certain degree of cytotoxic effect only at high concentrations, but the drug resistance of CCRF-VCR1000 to VCR can be effectively reversed.

Brassinolide inhibits the elimination of the medication by the P glucoprotein of the drug-resistant CCRF-VCR1000 strain cell membrane, while at the same time restoring the abnormal P53 protein expression level to nearly the level of sensitive cells. The downward adjustment of the abnormally expressed P53 may be one the mechanisms by which brassinolide reverses multi-drug resistance.

Key to Fig. 2

+brassinolide

Key to Fig. 3

Positive comparison

+brassinolide

P53 protein

P21 protein